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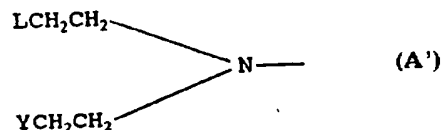
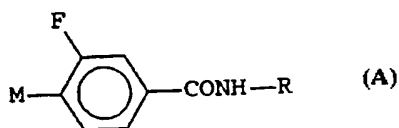
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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁵ : C07C 237/36, 229/60, A61K 31/195	A1	(11) International Publication Number: WO 94/25429 (43) International Publication Date: 10 November 1994 (10.11.94)
(21) International Application Number: PCT/GB94/00941 (22) International Filing Date: 3 May 1994 (03.05.94) (30) Priority Data: 9308957.1 30 April 1993 (30.04.93) GB (71) Applicant (for all designated States except US): CANCER RESEARCH CAMPAIGN TECHNOLOGY LIMITED [GB/GB]; Cambridge House, 6-10 Cambridge Terrace, Regent's Park, London NW1 4JL (GB). (72) Inventor; and (75) Inventor/Applicant (for US only): SPRINGER, Caroline, Joy [GB/GB]; Cancer Research Campaign Laboratory, The Institute of Cancer Research, 15 Cotswold Road, Sutton, Surrey SM2 5NG (GB). (74) Agents: BRASNETT, Adrian, Hugh et al.; J.A. Kemp & Co., 14 South Square, Gray's Inn, London WC1R 5LX (GB).	(81) Designated States: JP, US, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE). Published With international search report.	

(54) Title: 4-AMINO-FLUOROBENZAMIDES AND THEIR USE AS CYTOTOXIC PRODRUGS



(57) Abstract

The invention provides a compound which is a 3-fluorobenzamide of formula (A) wherein R-NH is the residue of an α -amino acid R-NH₂ or oligopeptide R-NH₂, and M is a nitrogen mustard group of formula (A') wherein Y and L, which may be the same or different in a molecule, are leaving groups; or a pharmaceutically acceptable salt thereof. The compounds are useful as prodrugs for treating cancer.

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4-AMINO-FLUOROBENZAMIDES AND THEIR USE AS CYTOTOXIC PRODRUGS

This invention relates to prodrugs, their use in therapy and a process for their preparation.

Over the years, many cytotoxic compounds have been discovered which are of potential use in cancer chemotherapy. Nitrogen mustards from one important family of such cytotoxic compounds. The clinical use of cytotoxic compounds in general and nitrogen mustards in particular has been limited because of the poor selectivity in the cytotoxic effect between tumour cells and normal cells.

One approach to overcome this problem has involved the development of so-called prodrugs which are derivatives of the cytotoxic drug, often a relatively simple derivative, whose cytotoxic properties are considerably reduced compared to those of the parent drug. Numerous proposals have been made for the administration of such prodrugs to patients under regimes whereby the prodrug is only converted to the cytotoxic drug in the region of the intended site of action.

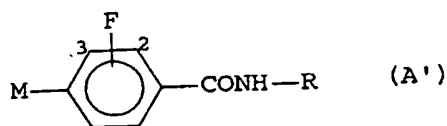
One particularly promising approach involves the conversion of the nitrogen mustard into a reaction product with an amino acid or oligopeptide to form a prodrug which can be converted to the parent nitrogen mustard at the site of intended action under the influence of an enzyme. This approach can be put into practice by the utilization of an antibody/enzyme conjugate in association with a prodrug. The antibody/enzyme conjugate is one formed from an antibody to a tumour-associated antigen and an enzyme that will convert the prodrug to the cytotoxic drug. In clinical practice, the antibody/enzyme conjugate is first administered to the patient

and is allowed to localise in the region of the tumour to be treated. The prodrug is then administered to the patient so that conversion of the prodrug to the cytotoxic drug is also localised in the region of the tumour to be treated under the influence of the localised enzyme. Such a system is described in our WO-A-88/07378, and is now called "antibody-directed enzyme prodrug therapy" (ADEPT).

Specific prodrugs that can be used in ADEPT are those based upon benzoic acid nitrogen mustards (WO-A-88/07378). The cytotoxic benzoic acid nitrogen mustard is a bifunctional alkylating agent and the activating effect of the ionised carboxyl group is masked in the prodrug by converting the carboxyl group into an amide by reaction with an α -amino acid, the preferred α -amino acid being glutamic acid. The relatively inactive prodrug can be activated to its corresponding benzoic acid at a tumour site by prior administration of a monoclonal antibody coupled to the enzyme carboxypeptidase G2 (CPG2). Benzoic acid nitrogen mustard prodrugs and their cytotoxic drugs are also described in Springer *et al.*, J. Med. Chem., (1990) 33, 677-681 and Springer *et al.*, Anti-Cancer Drug Design (1991) 6, 467-479.

It is desirable to release a very reactive drug at the tumour in ADEPT. It is therefore an advantage to have prodrugs and corresponding active drugs of high reactivities, so that high efficacy in vivo may be obtained.

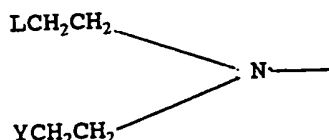
I have now synthesized 2- and 3-fluoro ring substituted benzoic acid nitrogen mustards of general formula (A')



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wherein R-NH is the residue of an α -amino acid R-NH₂ or oligopeptide R-NH₂, and M is a nitrogen mustard group of the formula

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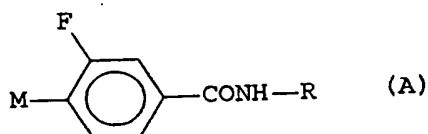


wherein Y and L, which may be the same or different in a molecule, are leaving groups; and pharmaceutically acceptable salts thereof. The F group may be at the 2- or 3- position relative to the -CONH-R group.

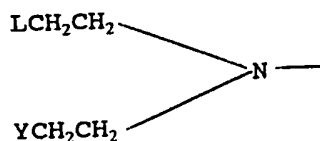
15 I have found that these compounds have surprising reactivities. Due to the strong inductive effect of fluorine, it would have been expected that a fluorine in the ring at position 2 or 3 would cause deactivation of the alkylating moiety, and that the inductive effect would be greater in the 3-position than in the 2-position. Thus, theoretically this would lead to the 3-fluoro compounds being less reactive than the 2-fluoro compounds. However, I found that although the 2-fluoro prodrugs and their corresponding drugs are deactivated as expected (i.e. less reactive than their non-fluorinated analogues), the 3-fluoro prodrugs and drugs are greatly activated (i.e. much more reactive than their non-fluorinated analogues). Further, all of the 3-fluoro but not all of the 2-fluoro prodrugs tested are good substrates for CPG 2.

WO 93/08288 discloses 2- and 3-fluoro ring substituted nitrogen mustard compounds, but these compounds contain an $-NH_2$ group in place of the protected carboxyl group of the present benzoic acid nitrogen mustard based prodrugs. The compounds containing an $-NH_2$ group are very reactive and the addition of a 3-fluoro group makes no significant difference to their reactivity, in contrast to the great increase in reactivity caused by the 3-fluoro group in the present compounds.

The present invention provides a compound which is a 3-fluorobenzamide of the formula (A)

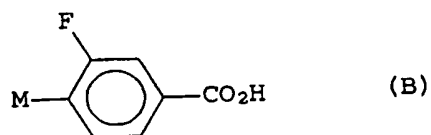


wherein $R-NH$ is the residue of an α -amino acid $R-NH_2$ or oligopeptide $R-NH_2$, and M is a nitrogen mustard group of the formula



wherein Y and L, which may be the same or different in a molecule, are leaving groups; or a pharmaceutically acceptable salt thereof.

The prodrug is converted into the active drug by cleavage of the amide bond between the residue of the α -amino acid $R-NH_2$ or oligopeptide $R-NH_2$ and the residue of the benzoic acid nitrogen mustard. The active drug has the formula (B)



5

wherein M is as defined above.

The prodrug is suitable for use in a method of treatment of the human or animal body by therapy, particularly a method of treatment of cancer. The invention includes a method of
10 treating a human or animal suffering from cancer, which method comprises administering to the patient a prodrug of the invention. The cancer may be any disease in which there is neoplastic cell growth, including leukemias and solid tumours (e.g. colorectal and ovarian tumours).

15 The prodrug may be selectively converted to the active drug by the enzyme component of an immunoglobulin/enzyme conjugate localised in the region of a tumour to be treated. Accordingly, the prodrug may be used in a method which comprises administering to a human or animal suffering from
20 cancer pharmaceutically effective amounts of

(i) an immunoglobulin/enzyme conjugate in which the immunoglobulin is specific for a tumour-associated antigen, and the enzyme will cleave the amide bond between the residue of the α -amino acid R-NH₂ or
25 oligopeptide R-NH₂ and the benzoic acid nitrogen mustard residue; and thereafter

(ii) the said prodrug.

Examples of suitable immunoglobulins and enzymes are given in WO-A-88/07378. The immunoglobulin may be an antibody

or a fragment of an antibody containing at least one of the antigen binding sites of the antibody. The antibody is preferably monoclonal but could be polyclonal. The antibody will generally be of the IgG class but other classes of antibody are not excluded. The antibody may be humanised, e.g. as described by Winter in EP-A-239 400. The antibody fragment is generally a Fab' or F(ab')₂ fragment. The enzyme is preferably a carboxypeptidase (e.g. bacterial carboxypeptidase G2 (CPG2)).

10 In the prodrug of the invention, the groups Y and L, which may be the same or different in a molecule, may for example be halo, mesyloxy or 4-tosyloxy. Preferably, Y and L are both mesyloxy, Y and L are both chloro, or Y is mesyloxy and L is chloro.

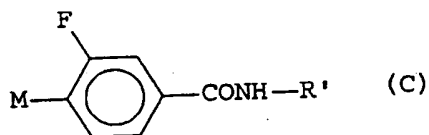
15 The amino acid or oligopeptide R-NH₂ is selected in order that the group R-NH of prodrugs of the invention may be removed in vivo under the influence of an enzyme. Glutamic acid and aspartic acid are suitable amino acids, although other α -amino carboxylic acids may also be of use. The amino acids are
20 preferably in the L configuration.

Examples of prodrugs of the invention are
3-fluoro-4-[bis-[2-(mesyloxy)ethyl]amino]benzoyl-L-glutamic acid,
3-fluoro-4-[(2-chloroethyl)[2-(mesyloxy)ethyl]amino]benzoyl-L-
25 glutamic acid,
3-fluoro-4-[bis(2-chloroethyl)amino]benzoyl-L-glutamic acid,
and pharmaceutically acceptable salts thereof.

The prodrugs of the invention may be produced by processes analogous to those described in WO-A-88/07378, WO-A-

90/02729 and WO-A-91/03460. The process of the present invention comprises deprotecting a compound of the formula (C)

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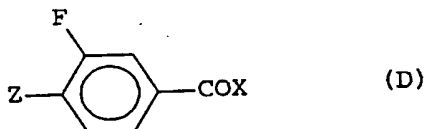
wherein M is as defined above, and R'-NH is the residue of an α -amino acid R'-NH₂ or oligopeptide R'-NH₂ containing at least one protected carboxylic acid group, and optionally converting the resulting compound of formula (A) as defined above into a pharmaceutically acceptable salt thereof. The compound of formula (C) is novel and forms part of the invention.

The at least one protected carboxylic acid group may, for example, be protected by an ethyl or tertiary butyl group. WO-A-88/07378 describes conventional methods of removing ethyl protecting groups which may be used in the present invention. In these methods, the ethyl protecting groups are removed by alkaline hydrolysis with aqueous sodium hydroxide followed by conversion of the resulting sodium salt into the free carboxylic acid using hydrochloric acid.

Preferably, the protecting groups are tertiary butyl. WO-A-90/02729 describes a suitable method of removing the tertiary butyl protecting groups. The tertiary butyl ester groups can be converted into free carboxylic acid groups by treatment with an acid, for example in a non-aqueous medium. Trifluoroacetic acid and formic acid are suitable acids. Removal of the tertiary butyl ester group can be carried out quite simply by maintaining the tertiary butyl ester in a

substantially non-aqueous solution together with trifluoroacetic acid at room temperature, e.g. 15 - 25°C. It is desirable to utilise an amount of trifluoroacetic acid that is at least equivalent to the tertiary butyl ester groups to be hydrolysed although the exact proportion of trifluoroacetic acid and the hydrolysis temperature are not critical, the use of lower temperatures and smaller proportions of trifluoroacetic acid serving merely to prolong the period of time necessary for total hydrolysis of the tertiary butyl ester groups to take place. Hydrolysis of tertiary butyl ester groups with trifluoroacetic acid under non-aqueous conditions proceeds almost quantitatively (>80%).

The compound of formula (C) may be obtained by reacting a compound of formula (D)

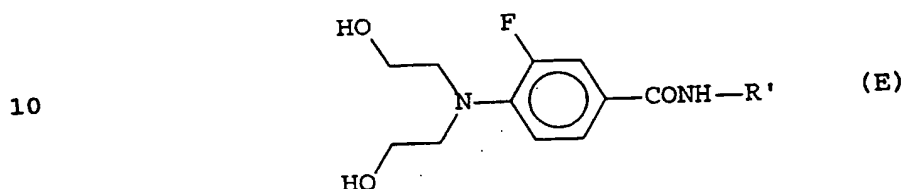


20 wherein X is hydroxy or halogen (e.g. chlorine) and Z is a group (e.g. NO₂) capable of being converted to a nitrogen mustard group M as defined above, with a protected α-amino acid R'-NH₂ or oligopeptide R'-NH₂ as defined above, followed by conversion of the group Z (e.g. through NH₂) to a group M as defined above.

25 The protected α-amino acids (e.g. t-butylated glutamic acid or aspartic acid) and oligopeptides may be obtained commercially (e.g. from Sigma Chemical Company Limited). Alternatively, they may be prepared by conventional means. For

example, glutamic acid may be reacted with t-butylacetate. The compounds of formula (D) may be obtained from 3F, 4NO₂ toluene which are commercially available (e.g. from Aldrich Chemical Company Limited) by the method of Jackman *et al.*, J. Med. Chem. 5 (1990) 33, 3067-3071 and Marsham *et al.*, *ibid* 3072-3078.

In a preferred method of producing the compound of formula (C), a compound of formula (E)

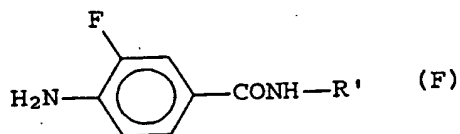


wherein R'-NH is as defined above, is reacted with a compound of formula



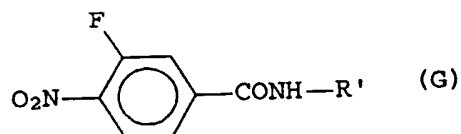
wherein A is a methyl or 4-tolyl group, and B is a halogen (e.g. chlorine). The reaction is suitably carried out in an organic solvent, e.g. pyridine.

The compound of formula (E) is preferably prepared by 20 reacting a compound of formula (F)



25 wherein R'-NH₂ is as defined above with ethylene oxide in a solvent, e.g. acetic acid.

The compound of formula (F) is preferably prepared by reducing a compound of formula (G)



5

wherein $R'-NH_2$ is as defined above. The reduction may, for example, be effected by catalytic transfer of the compound of formula (G), e.g. by ammonium formate in methanol in the presence of a Pd/C catalyst.

10

The compound of formula (G) may be obtained by reacting a compound of formula (D) with an α -amino acid or oligopeptide of formula $R'-NH_2$ as defined above. The reaction is carried out in a solvent such as CH_2Cl_2 .

The compounds of formulae (A), (C), (E), (F) and (G) may each be purified by conventional means, e.g. chromatography and/or crystallization. These compounds may each be prepared in the form of a salt. Pharmaceutically acceptable salts of the compound of formula (A) include base salts, e.g. those derived from an alkali metal (e.g. sodium) or alkaline earth metal (e.g. magnesium), and ammonium salts; and acid addition salts, including hydrochloride and acetate salts.

The invention includes a pharmaceutical composition comprising a prodrug of the invention and a pharmaceutically acceptable carrier or diluent. The invention also includes a kit comprising a prodrug or composition of the invention and an immunoglobulin/enzyme conjugate in which the immunoglobulin is specific for a tumour-associated antigen and the enzyme will cleave the amide bond between the residue of the α -amino acid $R-NH_2$ or oligopeptide $R-NH_2$ and the benzoic acid nitrogen

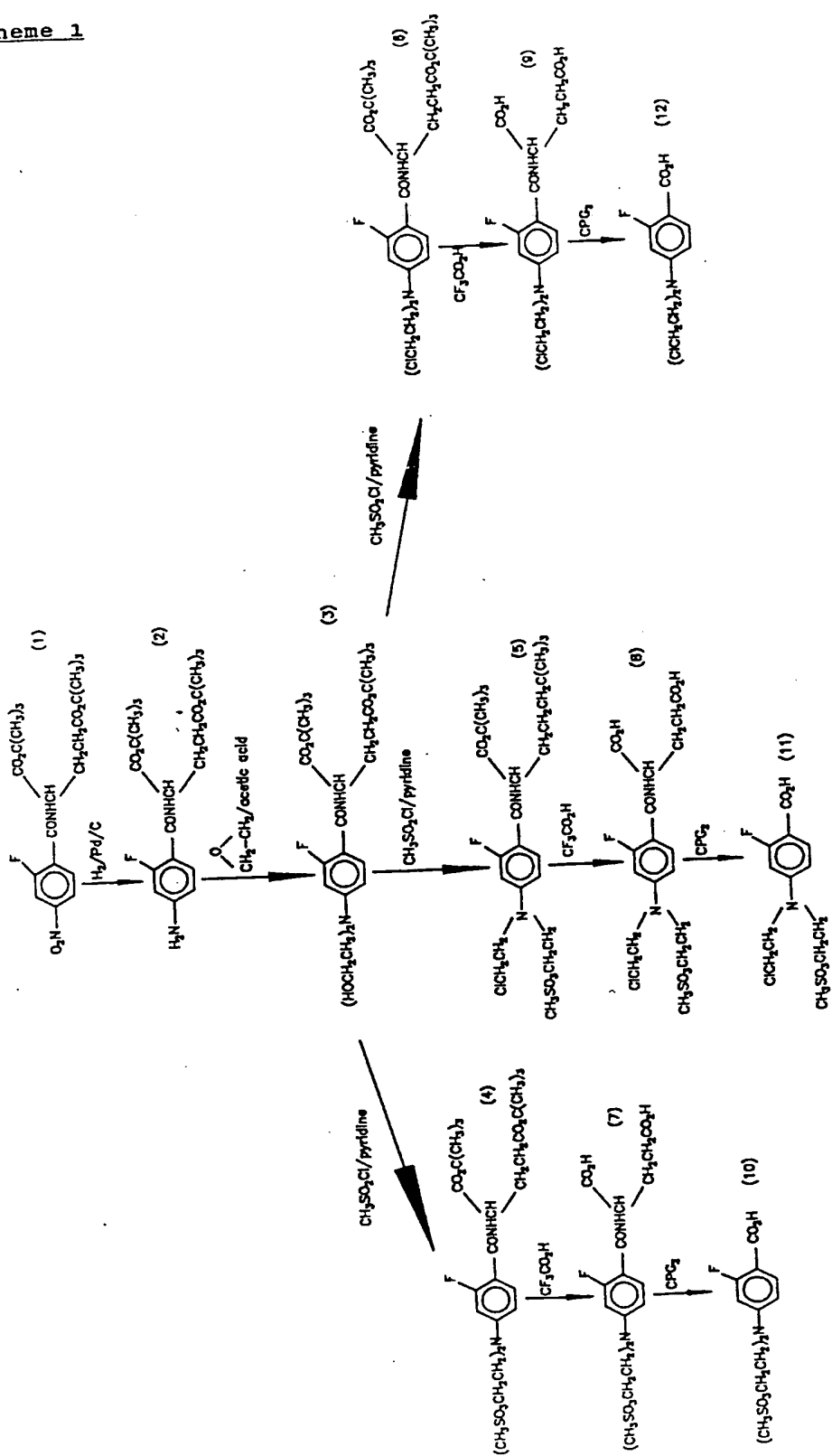
mustard residue.

The prodrug and immunoglobulin/enzyme conjugate will normally be administered parenterally, e.g. intravenously or intraperitoneally. Thus, the pharmaceutical composition of the invention is normally one which is suitable for parenteral (e.g. intravenous or intraperitoneal) administration. Such a composition conveniently contains the prodrug and isotonic saline or bicarbonate as diluent. The dose of the prodrug and conjugate will ultimately be at the discretion of the physician, who will take into account such factors as the age, weight and condition of the patient. Suitable doses of prodrug and conjugate are given in Bagshawe *et al.* Antibody, Immunoconjugates, and Radiopharmaceuticals (1991), 4, 915-922. A suitable dose of conjugate may be from 2000 to 200,000 enzyme units/m² (e.g. 20,000 enzyme units/m²) and a suitable dose of prodrug may be from 20 to 2000 mg/m² (e.g. 200 mg/m²).

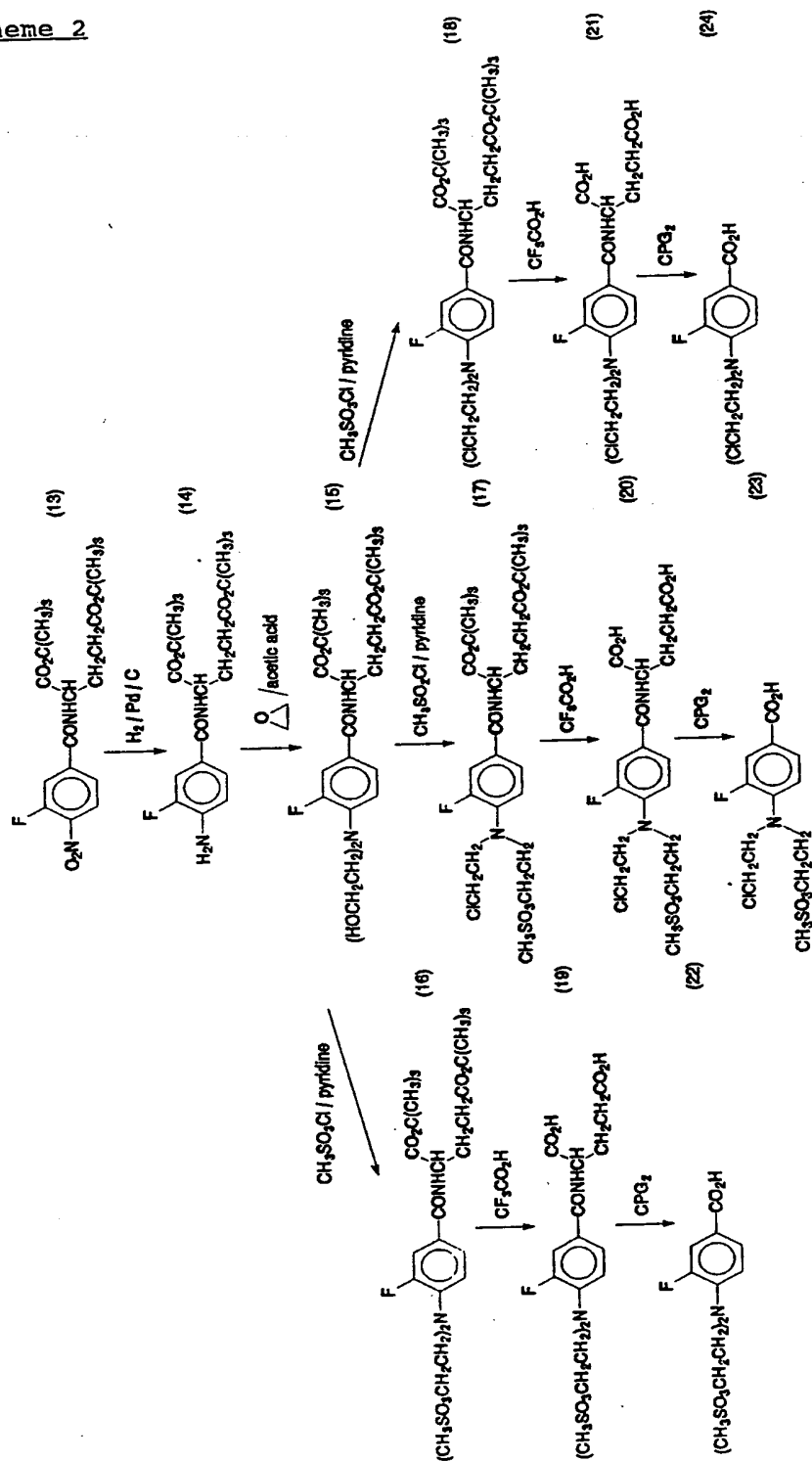
In order to secure maximum concentration of the conjugate at the site of desired treatment, it is normally desirable to space apart administration of the two components by at least 4 hours. The exact regime will be influenced by various factors including the nature of the tumour to be targeted and the nature of the prodrug. A typical regime is to administer the conjugate at 0 h, galactosylated clearing antibody at 24 h, and prodrug at 48 h. If no clearing antibody is used, it would generally be longer than 48 h before the prodrug could be injected.

The following Examples serve to illustrate the invention. The following Reaction Schemes 1 and 2 summarise the processes of Examples 1 and 2 respectively.

Scheme 1



Scheme 2



EXAMPLE 1: A REFERENCE EXAMPLE SHOWING THE SYNTHESIS OF 2-FLUORO PRODRUGS

Di-tert-butyl 2-fluoro,4-nitrobenzoyl-L-glutamate (1)

To a solution of di-tert butyl L-glutamate hydrochloride
5 (5.0 g, 16.9 mmol) in Et₃N (5.0 ml, 34.0 mmol) was added
dropwise a solution of 2-fluoro,4-nitro benzoyl chloride (3.5
g, 17.0 mmol) in CH₂Cl₂ (50 ml). Extractive workup gave an oil
(1); yield (6.8 g, 94%);

¹H NMR (Me₂SO-d₆) δ 1.40 (s, 9H, t-Bu), 1.43 (s, 9H, t-Bu), 1.95
10 (m, 2H, CH₂CH₂CO₂-t-Bu), 2.34 (t, 2H, J=7.4Hz, CH₂CH₂CO₂-t-Bu),
4.35 (m, 1H, CH), 7.85 (ddd, 1H, J_{H-6,H-3}=1.1, J_{H-6,F}=7.1, J_{H-6,H-5}=8.2Hz
H-6), 8.18 (m, 2H, H-3, H-5), 8.92 (d, 1H, J=7.46Hz, NH);
¹⁹F NMR (Me₂SO-d₆) δ -110.41 (dd, J_{F,H-3}-J_{F,H-6}=6.96Hz);

mass spectrum (FAB) m/z ([M+H⁺], 18), 315 (M-t-Bu₂, 100);

15 Anal: C₂₀H₂₇N₂O₇F-0.04CH₂Cl₂ requires C-55.99, H-6.35, N-6.52, F-
4.42, Cl-0.66, found C-55.64, H-6.08, N-6.41, F-4.44, Cl-0.31.
(The presence of CH₂Cl₂ noted in the elemental analysis was
confirmed by NMR).

20 Di-tert-butyl 2-fluoro,4-aminobenzoyl-L-glutamate (2)

Catalytic transfer reduction of the nitro compound (1)
(5.8 g, 13.6 mmol) in MeOH (60 ml) with ammonium formate (4.5
g, 71.5 mmol) over Pd/C (10%) gave the amine (2) as an oil;
yield (5.2 g, 96%);

25 ¹H NMR (Me₂SO-d₆) δ 1.38 (s, 9H, t-Bu), 1.41 (s, 9H, t-Bu), 1.95
(m, 2H, CH₂CH₂CO₂-t-Bu), 2.29 (t, 2H, J=8.13Hz, CH₂CH₂CO₂-t-Bu),
4.31 (m, 1H, CH), 5.98 (s, 2H, NH₂), 6.30 (dd, 1H, J_{H-3,F}=14.33Hz,
H-3), 6.40 (dd, 1H, J_{H-5,H-6}=8.57Hz, H-5), 7.42 (dd, 1H, J_{H-6,H-5}
=8.70, J_{H-6,F}=17.46 Hz, H-6), 7.69 (dd, 1H, J_{H-N,H-C}=6.75, J_{H-N}

$\nu_{\text{F}}=13.93\text{Hz}$, NH);

^{19}F NMR ($\text{Me}_2\text{SO}-d_6$) δ -112.23 (ddd);

mass spectrum (FAB) m/z (397 [$\text{M}+\text{H}^+$], 100), 341 (M-t-Bu, 45);

Anal: $\text{C}_{20}\text{H}_{29}\text{N}_2\text{O}_5\text{F}-0.5\text{MeOH}$ requires C-59.69, H-7.58, N-6.79, F-4.61, found C-59.84, H-7.48, N-7.02, F-4.79.

Di-tert-butyl 2-fluoro,4-[Bis(2-hydroxyethyl)amino]benzoyl-L-glutamate (3)

Amine (2) (1.6 g, 4.0 mmol) in HOAc (10 ml) was stirred with ethylene oxide (13.0 ml, 260 mmol) at room temperature for 112 h. The product was partitioned between CH_2Cl_2 and H_2O . The organic phase was separated, washed with H_2O , dried (Na_2SO_4), and evaporated to dryness. The crude oil was chromatographed on silica gel, eluting with EtOAc- CH_2Cl_2 to give an oil (3); yield (1.0 g, 49%).

^1H NMR ($\text{Me}_2\text{SO}-d_6$) δ 1.39 (s, 9H, t-Bu), 1.42 (s, 9H, t-Bu), 1.93 (m, 2H, $\text{CH}_2\text{CH}_2\text{CO}_2$ -t-Bu), 2.29 (t, 2H, $J=7.69\text{Hz}$, $\text{CH}_2\text{CH}_2\text{CO}_2$ -t-Bu), 3.46 (d, 4H, $J=5.23\text{Hz}$, $(\text{HOCH}_2\text{CH}_2)_2$), 3.55 (t, 4H, $J=4.80\text{Hz}$, $(\text{HOCH}_2\text{CH}_2)_2$), 4.34 (m, 1H, CH), 4.75 (t, 2H, $J=4.67\text{Hz}$, $(\text{OH})_2$), 6.50 (dd, 1H, $J_{\text{H-3,F}}=17.09\text{Hz}$, H-3), 6.57 (dd, 1H, $J_{\text{H-5,H-6}}=8.97\text{Hz}$, H-5), 7.33 (dd, 1H, $J_{\text{H-6,F}}=9.1\text{Hz}$, H-6), 7.69 (dd, 1H, $J_{\text{H-N,H-C}}=7.11$, $J_{\text{H-N,F}}=14.07\text{ Hz}$, NH);

^{19}F NMR ($\text{Me}_2\text{SO}-d_6$) δ -111.03 (ddd);

mass spectrum (FAB) m/z (485 [$\text{M}+\text{H}^+$], 4), 226 (M-glutBu₂, 100);

Anal: $\text{C}_{24}\text{H}_{37}\text{N}_2\text{O}_7\text{F}-0.5\text{EtOAc}$ requires C-59.07, H-7.82, N-5.30, F-3.59, found C-59.23, H-7.71, N-5.20, F-3.32. (The presence of EtOAc noted in the elemental analysis was confirmed by NMR).

Di-tert-butyl 2-fluoro, 4-[Bis(2-(mesyloxy)ethyl)amino]benzoyl-L-glutamate, (4)

Di-tert-butyl 2-fluoro, 4-[(2-chloroethyl)[2-(mesyloxy)ethyl](2-fluoro)amino]benzoyl-L-glutamate (5)

5 Di-tert-butyl 2-fluoro, 4-[Bis(2-chloroethyl)amino]benzoyl-L-glutamate (6)

A solution of (3) (1.4 g, 2.9 mmol) in pyridine (4.5 ml) was stirred with methane sulphonyl chloride (0.9 ml, 1.8 mmol) at 0°C for 20 min followed by 80°C for 20 min. The reaction mixture was partitioned between EtOAc and citric acid (10%).
10 The organic phase was separated, washed with H₂O, dried (Na₂SO₄), and evaporated to dryness. The concentrate contained three reaction products, each of which gave a positive colour with the Epstein reagent. The mixture was chromatographed on
15 silica gel. The slowest eluting was the 2-fluoro, bis[2-(mesyloxy)ethyl-derivative, a solid (4); mp 90-92°C, yield (0.10 g, 5%);
¹H NMR (Me₂SO-d₆) δ 1.38 (s, 9H, t-Bu), 1.42 (s, 9H, t-Bu), 1.94 (m, 2H, CH₂CH₂CO₂-t-Bu), 2.30 (t, 2H, J=7.79Hz, CH₂CH₂CO₂-t-Bu),
20 3.16 (s, 6H, (CH₃SO₃)₂), 3.80 (t, 4H, J=5.21Hz, (CH₃SO₃CH₂CH₂)₂), 4.33 (t, 5H, J=5.30Hz, (CH₃SO₃CH₂CH₂)₂ & CH), 6.68 (dd, 2H, J_{H-5,H-6}=8.14, J_{H-3,F}=15.27Hz, H-3, H-5), 7.54 (dd, 1H, J_{H-6,F}=9.1Hz, H-6) 7.90 (dd, 1H, J_{H-N,H-C}=5.31, J_{H-N,F}=12.52Hz, NH); ¹⁹F NMR (Me₂SO-d₆) δ -100.52 (dd);
25 mass spectrum (FAB) m/z (641 [M+H⁺], 12), 382 (M-glutBu₂, 100);
Anal: C₂₆H₄₁N₂O₁₁FS₂ requires C-48.73, H-6.45, N-4.37, F-2.97, S-10.01, found C-48.62, H-6.26, N-4.32, F-2.73, S-10.09.

Eluting second was an oil, the 2-fluoro, (2-chloroethyl)[2-(mesyloxy)ethyl] derivative (5); yield (0.58 g, 34%);

- ¹H NMR (Me₂SO-d₆) δ 1.38 (s, 9H, t-Bu), 1.42 (s, 9H, t-Bu), 1.93 (m, 2H, CH₂CH₂CO₂-t-Bu), 2.30 (t, 2H, J=7.82Hz, CH₂CH₂CO₂-t-Bu), 3.15 (s, 3H, CH₃SO₃), 3.77 (s, 4H, ClCH₂CH₂), 3.82 (t, 2H, J=5.18Hz, CH₃SO₃CH₂CH₂), 4.32 (t, 3H, J=5.17Hz, CH₃SO₃CH₂CH₂ & CH), 6.66 (m, 2H, H-3, H-5), 7.55 (dd, 1H, J_{H-6,H-5}=8.79, J_{H-6,F}=9.2Hz, H-6), 7.89 (dd, 1H, J_{H-N,H-C}=5.64, J_{H-N,F}=12.82Hz, NH);
- ¹⁹F NMR (Me₂SO₂-d₆) δ -110.45 (m);
- mass spectrum (FAB) m/z (581 [M+M⁺], 14), 322 (M-glutBu₂, 100);
- Anal: C₂₅H₃₈N₂O₈FCls requires C-51.67, H-6.59, N-4.82, F-3.27, Cl-6.10, S-5.52, found C-51.92, H-6.53, N-4.82, F-3.16, Cl-6.06, S-5.48.

15

The fastest eluting, 2-fluoro, bis(2-chloroethyl) derivative was a solid (6); mp 104-106°C, yield (0.53 g, 34%);

- ¹H NMR (Me₂SO-d₆) δ 1.38 (s, 9H, t-Bu), 1.42 (s, 9H, t-Bu), 1.96 (m, 2H, CH₂CH₂CO₂-t-Bu), 2.29 (t, 2H, J=7.79Hz, CH₂CH₂CO₂-t-Bu), 3.78 (dt, 8H, J=5.29Hz (ClCH₂CH₂)₂), 4.35 (m, 1H, CH), 6.65 (m, 2H, H-3, H-5), 7.55 (dd, 1H, J_{H-6,H-5}=9.1, J_{H-6,H-5}=9.4Hz, H-6), 7.88 (dd, 1H, J_{H-N,H-C}=5.53, J_{H-N,F}=12.84Hz, NH)
- ¹⁹F NMR (Me₂SO-d₆) δ -110.55 (ddd, J_{F,H-N}=11.26, J_{F,H-3}=14.07, J_{F,H-6}=16.32Hz);

- mass spectrum (FAB) m/z (521 [M+H⁺], 16), 262 (M-glutBu₂, 100)
- Anal: C₂₄H₃₅N₂O₅Cl₂ requires C-55.28, H-6.77, N-5.37, F-3.64, Cl-13.60, found C-55.43, H-6.82, N-5.39, F-3.62, Cl-13.91.

Preparation of diacids - General Method

Compound (4) (0.13 g, 0.20 mmol), (5) (0.21 g, 0.36 mmol), or (6) (0.20 g, 0.38 mmol) was suspended in TFA (4-8% w/v) and stirred for 40 min at room temperature. The solvent was removed under reduced pressure and the remaining oil was diluted with ethyl acetate (1 ml) which was evaporated. This latter step was repeated 5-20 times. Compound (7); yield (0.12 g, 100%), 2-fluoro, 4-[bis-[2-(mesyloxy)ethyl]amino]benzoyl-L-glutamic acid, was obtained as a pure product from (4);

¹H NMR (Me₂SO-d₆) δ 2.05 (m, 2H, CH₂CH₂CO₂H), 2.32 (t, 2H, J=7.61Hz, CH₂CH₂CO₂H), 3.16 (s, 6H, (CH₃SO₃)₂), 3.81 (t, 4H, J=4.98Hz, (CH₃SO₃CH₂CH₂)₂), 4.33 (t, 4H, J=5.30Hz, (CH₃SO₃CH₂CH₂)₂), 4.41 (t, 1H, J=4.16Hz, CH), 6.69 (m, 2H, H-3, H-5), 7.58 (dd, 1H, J_{H-6,F}=9.1, J_{H-6,H-5}=9.3Hz, H-6), 7.89 (dd, 1H, J_{H-N,H-C}=6.04, J_{H-N}

¹⁵ F=12.08Hz, NH);

¹⁹F NMR (Me₂SO-d₆) δ -110.35(ddd, J_{F,H-3}=14.52Hz);

mass spectrum (FAB) m/z (529[M+H⁺], 12), 382 (M-glu, 100);

Accurate mass Expected 529.0961 found +2.0 ppm;

Anal: C₁₈H₂₅N₂O₁₁FS₂-0.40TFA-0.30EtOAc requires C-39.99, H-4.67, N-4.67, F-6.96, S-10.68, found C-39.62, H-4.50, N-4.64, F-6.57, S-10.40. (The presence of EtOAc and TFA noted in the elemental analysis was confirmed by NMR).

This compound reacted positively with the Epstein spray reagent.

25

Compound (8); yield (0.17 g, 92%), 2-fluoro, 4-[(2-chloroethyl)[2-(mesyloxy)ethyl]amino]benzoyl-L-glutamic acid, was similarly obtained as an oil from (5);

30

- ¹H NMR (Me₂SO-d₆) δ 2.02 (m, 2H, CH₂CH₂CO₂H), 2.32 (t, 2H, J=7.53Hz, CH₂CH₂CO₂H), 3.15 (s, 3H, CH₃SO₃), 3.77 (s, 4H, ClCH₂CH₂), 3.82 (t, 2H, J=5.11Hz, CH₃SO₃CH₂CH₂), 4.32, (t, 2H, J=5.31Hz, CH₃SO₃CH₂CH₂), 4.40 (q, 1H, J=4.54Hz, CH), 6.67 (m, H-3, H-5), 7.57 (dd, 1H, J_{H-6,F}=9.1, J_{H-6,H-5}=9.4Hz, H-6), 7.88 (dd, 1H, J_{H-N,H-C}=6.53, J_{H-N,F}=13.06Hz, NH);
- ¹⁹F NMR (Me₂SO-d₆) δ -110.35 (ddd, J_{F,H-3}=16.19Hz);
- mass spectrum (FAB) m/z (469 [M+H⁺], 8), 322 (M-glu, 100);
- Accurate mass Expected 469.0847 found +3.2 ppm;
- 10 Anal: C₁₇H₂₂N₂O₈FCls-0.26TFA-0.15EtOAc requires C-42.52, H-4.62, N-5.48, F-6.60, Cl-6.93, S-6.27, found C-42.12, H-4.68, N-5.13, F-6.20, Cl-6.67, S-6.0. (The presence of EtOAc and TFA, noted in the elemental analysis was confirmed by NMR).
- This compound reacted positively with the Epstein spray
- 15 reagent.

Compound (9); yield (0.17 g, 97%), 2-fluoro, 4-[bis(2-chloroethyl)amino]benzoyl-L-glutamic acid, was likewise obtained as an oil from (6);

- 20 ¹H NMR (Me₂SO-d₆) δ 1.98 (m, 2H, CH₂CH₂CO₂H), 2.33 (t, 2H, J=7.70Hz, CH₂CH₂CO₂H), 3.78 (dt, 8H, (ClCH₂CH₂)₂), 4.41 (m, 1H, CH), 6.65 (m, 2H, H-3, H-5), 7.58 (dd, 1H, J_{H-6,H-5}=8.83, J_{H-6,F}=9.1Hz, H-6), 7.85 (dd, 1H, J_{H-N,H-C}=5.53, J_{H-N,F}=12.84Hz, NH);
- ¹⁹F NMR (Me₂SO-d₆) δ -110.43 (ddd, J_{F,H-3}=15.27Hz);
- 25 mass spectrum (FAB) m/z (409[M+H⁺]3), 262 (M-glu, 100);
- Accurate mass expected 409.0733 found +3.7 ppm;
- Anal: C₁₆H₁₉N₂O₅FCls-0.40TFA requires C-44.36, H-4.30, N-6.16, F-9.19, Cl-15.58, found C-44.59, H-4.29, N-5.83, F-8.81, Cl-15.58. (The presence of TFA noted in the elemental analysis

was confirmed by NMR).

This compound reacted positively with the Epstein spray reagent.

5 EXAMPLE 2: SYNTHESIS OF 3-FLUORO PRODRUGS

Di-tert-butyl 3-fluoro,4-nitrobenzoyl-L-glutamate (13)

To a solution of di-tert butyl L-glutamate hydrochloride (20.0 g, 67.6 mol) in Et₃N (19 ml, 136.0 mmol) was added dropwise a solution of 3-fluoro,4-nitro benzoyl chloride (13.8 g, 68.0 mmol) in CH₂Cl₂ (300 ml). Extractive workup gave an oil (13); yield (28.0 g, 97%);

¹H NMR (Me₂SO-d₆) δ 1.40 (s, 9H, t-Bu), 1.42 (s, 9H, t-Bu), 1.99 (m, 2H, CH₂CH₂CO₂-t-Bu), 2.36 (t, 2H, J=7.45Hz, CH₂CH₂CO₂-t-Bu), 4.35 (m, 1H, CH), 7.92 (dd, 1H, J_{H-6,H-5}=7.56Hz, H-6), 8.01 (dd, 1H, J_{H-2,F}=11.97Hz, H-2), 8.29 (dd, 1H, J_{H-5,H-6}= 7.83, J_{H-5,F}=16.10Hz, H-5), 8.97 (d, 1H, J=7.42Hz, NH);

¹⁹F NMR (Me₂SO-d₆) δ -117.98 (dd, J_{F,H-2}= 7.32, J_{F,H-5}=17.8 Hz);

mass spectrum (CI) m/z 427 ([M+H⁺], 100);

Anal: C₂₀H₂₇N₂O₇F-0.25CH₂Cl₂ requires C-54.33, H-6.19, N-6.26, F-4.24, Cl-3.96, found C-54.22, H-6.38, N-6.05, F-4.34, Cl-4.15. (The presence of CH₂Cl₂ noted in the elemental analysis was confirmed by NMR).

Di-tert-butyl 3-fluoro, 4-aminobenzoyl-L-glutamate (14)

25 Catalytic transfer reduction of the nitro compound (13) (7.5 g, 17.5 mmol) in MeOH (60 ml) with ammonium formate (5.8 g, 91.6 mmol) on Pd/C (10%) gave the amine (14) as an oil (6.9 g, 99%)

¹H NMR (Me₂SO-d₆) δ 1.39 (s, 9H, t-Bu), 1.40 (s, 9H, t-Bu), 1.97

- (m, 2H, $\text{CH}_2\text{CH}_2\text{CO}_2\text{-t-Bu}$), 2.31 (t, 2H, $J=7.44\text{Hz}$, $\text{CH}_2\text{CH}_2\text{CO}_2\text{-t-Bu}$), 4.28 (m, 1H, CH), 5.71 (s, 2H, NH_2), 6.77 (dd, 1H, $J_{\text{H-5,H-6}}=8.77$, $J_{\text{H-5,F}}=17.43\text{Hz}$, H-5), 7.49 (dd, 1H, $J_{\text{H-6,H-5}}=8.32\text{Hz}$, H-6), 7.56 (dd, 1H, $J_{\text{H-2,F}}=12.79\text{Hz}$, H-2), 8.19 (d, 1H, $J=7.55\text{Hz}$, NH);
- 5 ^{19}F NMR ($\text{Me}_2\text{SO-d}_6$) δ -135.56 (dd, $J_{\text{F,H-2}}=12.21$, $J_{\text{F,H-5}}=20.51\text{ Hz}$);
 mass spectrum (FAB) m/z 396 ($[\text{M}+\text{H}^+]$, 5) 138 (M-glu, 100);
 Anal: $\text{C}_{20}\text{H}_{29}\text{N}_2\text{O}_5\text{F}$ requires C-60.59, H-7.37, N-7.07, F-4.79, found C-60.50, H-7.34, N-7.09, F-4.69.
- 10 Di-tert-butyl 3-fluoro,4-[Bis(2-hydroxyethyl)amino]benzoyl-L-glutamate (15)
- Amine (14) (5.3 g, 13.4 mmol) in HOAc (30 ml) was stirred with ethylene oxide (60 ml, 1.2 mol) at room temperature for 336 h. The solvent was partitioned between EtOAc and H_2O . The organic phase was separated, washed with H_2O , dried (Na_2SO_4), and evaporated to dryness. The crude oil was chromatographed on silica gel, eluting with EtOAc- CH_2Cl_2 to give the pure oil (15); yield (3.3 g, 51%)
- 15 ^1H NMR ($\text{Me}_2\text{SO-d}_6$) δ 1.39 (s, 9H, t-Bu), 1.41 (s, 9H, t-Bu), 1.97 (m, 2H, $\text{CH}_2\text{CH}_2\text{CO}_2\text{-t-Bu}$), 2.32 (t, 2H, $J=7.42\text{Hz}$, $\text{CH}_2\text{CH}_2\text{CO}_2\text{-t-Bu}$), 3.43 (t, 4H, $J=5.93\text{Hz}$, $(\text{HOCH}_2\text{CH}_2)_2$), 3.54 (d, 4H, $J=5.46\text{Hz}$, $(\text{HOCH}_2\text{CH}_2)_2$), 4.31 (m, 1H, CH), 4.67 (s, 2H, $(\text{OH})_2$), 6.99, (dd, 1H, $J_{\text{H-5,H-6}}=8.86$, $J_{\text{H-5,F}}=17.84\text{Hz}$ H-5), 7.60 (dd, 2H, $J_{\text{H-6,H-5}}=9.56$, $J_{\text{H-2,F}}=14.26\text{Hz}$ H-6, H-2) 8.30 (d, 1H, $J=7.48\text{Hz}$, NH);
- 20 ^{19}F NMR ($\text{Me}_2\text{SO-d}_6$) δ -124.31 (dd, $J_{\text{F,H-2}}=11.63$, $J_{\text{F,H-5}}=17.08\text{Hz}$);
 mass spectrum (FAB) m/z (485 $[\text{M}+\text{H}^+]$, 22), 226 (M-glutBu, 100);
 Anal: $\text{C}_{24}\text{H}_{37}\text{N}_2\text{O}_7\text{F}$ -1.1EtOAc requires C-58.66, H-7.94, N-4.82, F-3.27, found C-58.31, H-7.83, N-5.18, F-3.49. (The presence of EtOAc noted in the elemental analysis was confirmed by NMR).

Di-tert-butyl 3-fluoro,4-[Bis(2-(mesyloxy)ethyl)amino]benzoyl-L-glutamate (16)

Di-tert-butyl 3-fluoro,4-[(2-chloroethyl)[2-(mesyloxy)ethyl](2-fluoro)amino]benzoyl-L-glutamate (17)

5 Di-tert-butyl 3-fluoro,4-[Bis(2-chloroethyl)amino]benzoyl-L-glutamate (18)

A solution of (15) (0.67 g, 1.4 mmol) in pyridine (3 ml) was stirred with methane sulphonyl chloride (0.6 ml, 7.7 mmol) at 0°C for 20 min followed by 80°C for 15 min. The reaction mixture was partitioned between EtOAc and citric acid (10%). The organic phase was separated, washed with H₂O, dried (Na₂SO₄), and evaporated to dryness. The concentrate contained three reaction products, each of which gave a positive colour reaction with the Epstein reagent. The mixture was chromatographed on silica gel. The slowest eluting oil was the 3-fluoro, bis[2-(mesyloxy)ethyl-derivative, as the oil (16); yield (0.31 g, 35%);

¹H NMR (Me₂SO-d₆) δ 1.39 (s, 9H, t-Bu), 1.41 (s, 9H, t-Bu), 1.98 (m, 2H, CH₂CH₂CO₂-t-Bu), 2.32 (t, 2H, J=7.42Hz, CH₂CH₂CO₂-t-Bu), 3.12 (s, 6H, (CH₃SO₃)₂), 3.72 (t, 4H, J=5.41Hz, CH₃SO₃CH₂CH₂)₂, 4.30 (t, 5H, J=5.32Hz, (CH₃SO₃CH₂CH₂)₂ & CH), 7.16 (dd, 1H, J_{H-5,H-6}=8.79, J_{H-5,F}=8.8Hz, H-5) 7.67 (dd, 2H, J_{H-2,F}=13.81Hz, H-2, H-6), 8.43 (d, 1H, J=7.56Hz, NH);

¹⁹F NMR (Me₂SO-d₆) δ -122.69 (dd);

25 mass spectrum (FAB) m/z (641 [M+H⁺], 15), 382 (M-glutBu₂, 100);

Anal: C₂₆H₄₁N₂O₁₁FS₂-0.6EtOAc requires C-49.18, H-6.66, N-4.04, F-2.74, S-9.25, found C-48.92, H-6.59, N-4.00, F-2.85, S-8.93.

(The presence of EtOAc noted in the elemental analysis was confirmed by NMR).

Eluting second was the 3-fluoro, (2-chloroethyl)[2-(mesyloxy)ethyl] derivative, as the oil (17); yield (0.29 g, 37%);

- ¹H NMR (Me₂SO-d₆) δ 1.39 (s, 9H, t-Bu), 1.41 (s, 9H, t-Bu), 1.99 (m, 2H, CH₂CH₂CO₂-t-Bu), 2.32 (t, 2H, J=7.37Hz, CH₂CH₂CO₂-t-Bu), 3.12 (s, 3H, CH₃SO₃), 3.71 (s, 6H, ClCH₂CH₂ + CH₃SO₃CH₂CH₂), 4.30 (t, 3H, J=5.29Hz, CH₃SO₃CH₂CH₂ + CH), 7.13 (dd, 1H, J_{H-5,H-6}=8.81, J_{H-5,F}=9.0Hz, H-5), 7.66 (dd, 2H, J_{H-2,F}=14.58Hz, H-2, H-6), 8.41 (d, 1H, J=7.54Hz, NH);
- ¹⁹F NMR (Me₂SO-d₆) δ -123.40 (m);
- mass spectrum (FAB) m/z (581 [M+H⁺], 30), 322 (M-glutBu₂, 100);
- Anal: C₂₅H₃₈N₂O₈FCIS requires C-51.67, H-6.59, N-4.82, F-3.27, Cl-6.10 S-5.52, found C-51.29, H-6.60, N-4.56, F-3.18, Cl-5.74, S-5.29.

15

The fastest eluting, 3-fluoro, bis(2-chloroethyl) derivative was the solid (18), mp 100-103°C; yield (0.11 g, 15%);

- ¹H NMR (Me₂SO-d₆) δ 1.39 (s, 9H, t-Bu), 1.41 (s, 9H, t-Bu), 2.01 (m, 2H, CH₂CH₂CO₂-t-Bu), 2.33 (t, 2H, J=7.34Hz, CH₂CH₂CO₂-t-Bu), 3.72 (s, 8H, (ClCH₂CH₂)₂), 4.32, (m, 1H, CH), 7.11 (dd, 1H, J_{H-5,H-6}=8.86, J_{H-5,F}=9.1Hz, H-5), 7.65 (m, 2H, H-2, H-6), 8.40 (d, 1H, J=7.35Hz, NH);
- ¹⁹F NMR (Me₂SO-d₆) δ -123.83 (dd, J_{F,H-2}=14.8 Hz);
- mass spectrum (FAB) m/z (521 (M+H⁺), 19), 262 (M-glutBu₂, 100);
- Anal: C₂₄H₃₅N₂O₅Cl₂-0.5H₂O requires C-54.34, H-6.84, N-5.28, F-3.58, Cl-13.37, found C-54.71, H-6.61, N-5.31, F-3.64, Cl-13.54.

Preparation of diacids - General method

Compound (16) (0.10 g, 0.16 mmol), (17) (0.08 g, 0.13 mmol), or (18) (0.06 g, 0.11 mmol) was suspended in TFA (4% w/v) and stirred for 40 min at room temperature. The acid was removed under reduced pressure and the remaining oil was diluted with ethyl acetate (1 ml) which was evaporated. This latter step was repeated 5-6 times. Compound (19); yield (0.09 g, 91%) 3-fluoro, 4-[bis-(2-mesyloxy)ethyl]amino]benzoyl-L-glutamic acid, was obtained as a pure product from (16);

10 ¹H NMR (Me₂SO-d₆) δ 1.99 (m, 2H, CH₂CH₂CO₂H), 2.35 (t, 2H, J=7.45Hz, CH₂CH₂CO₂H), 3.13 (s, 6H, (CH₃SO₃)₂), 3.72 (t, 4H, J=5.34Hz, (CH₃SO₃CH₂CH₂)₂), 4.31 (t, 4H, J=5.16Hz, (CH₃SO₃CH₂CH₂)₂), 4.39 (m, 1H, CH), 7.16 (dd, 1H, J_{H-5,H-6}=8.59, J_{H-5,F}=18.03Hz, H-5), 7.68 (dd, J_{H-6,H-5}=8.91, J_{H-2,F}=15.29Hz, H-2, H-6), 8.45 (d, 1H, J=7.69Hz, NH);

15 ¹⁹F NMR (Me₂SO-d₆) δ -122.54 (m);
mass spectrum (FAB) m/z (529[M+H⁺], 45) 382 (M-glu, 100);
Accurate mass expected 529.0961 found +5.4ppm;
Anal: C₁₈H₂₅N₂O₁₁FS₂-0.22TFA-0.21EtOAc requires C-40.47, H-4.74, N-4.90, F-5.51, S-11.21, found C-40.87, H-4.76, N-4.75, F-5.85, S-10.98. (The presence of EtOAc and TFA, noted in the

20 elemental analysis was confirmed by NMR).
This compound reacted positively with the Epstein spray reagent.

25 Compound (20); yield (0.06 g, 91%), 3-fluoro, 4-[(2-chloroethyl)[2-(mesyloxy)ethyl]amino]benzoyl-L-glutamic acid, was similarly obtained as an oil from (17);

¹H NMR (Me₂SO-d₅) δ 2.00 (m, 2H, CH₂CH₂CO₂H), 2.35 (t, 2H, J=7.43Hz, CH₂CH₂CO₂H), 3.13 (s, 3H, CH₃SO₃), 3.73 (s, 6H, ClCH₂CH₂

+ $\text{CH}_3\text{SO}_3\text{CH}_2\text{CH}_2$), 4.31 (t, 2H, $J=5.40\text{Hz}$, $\text{CH}_3\text{SO}_3\text{CH}_2\text{CH}_2$), 4.39 (m, 1H, CH), 7.15 (dd, 1H, $J_{\text{H-5,H-6}}=8.81$, $J_{\text{H-5,F}}=18.24\text{Hz}$, H-5), 7.68 (dd, 2H, $J_{\text{H-2,F}}=14.75\text{Hz}$, H-2, H-6), 8.45 (d, 1H, $J=7.64\text{Hz}$, NH);
 ^{19}F NMR ($\text{Me}_2\text{SO}-d_6$) δ -123.19 (dd, $J_{\text{F,H-2}}=11.46$, $J_{\text{F,H-5}}=14.12\text{Hz}$);
5 mass spectrum (FAB) m/z (469 [$\text{M}+\text{H}^+$], 10), 322 (M-glu, 100);
Accurate mass expected 469.0847 found +4.9ppm;
Anal: $\text{C}_{17}\text{H}_{22}\text{N}_2\text{O}_8\text{FCIS}-0.20\text{TFA}-0.21\text{EtOAc}$ requires C-42.94, H-4.72, N-5.49, F-5.96, Cl-6.95, S-6.28, found C-43.34, H-4.79, N-5.16, F-5.95, Cl-6.82, S-5.89. (The presence of EtOAc and TFA noted
10 in the elemental analysis was confirmed by NMR).
This compound reacted positively with the Epstein spray reagent.

Compound (21); yield (0.05 g, 97%), 3-fluoro, 4-[bis(2-
15 chloroethyl)amino] benzoyl-L-glutamic acid, was likewise
obtained as an oil from (18):
 ^1H NMR ($\text{Me}_2\text{SO}-d_6$) δ 2.00 (m, 2H, $\text{CH}_2\text{CH}_2\text{CO}_2\text{H}$), 2.35 (t, 2H, $J=7.48\text{Hz}$, $\text{CH}_2\text{CH}_2\text{CO}_2\text{H}$), 3.73 (s, 8H, $(\text{ClCH}_2\text{CH}_2)_2$), 4.41 (m, 1H, CH), 7.12 (dd, 1H, $J_{\text{H-5,H-6}}=8.78$, $J_{\text{H-5,F}}=18.17\text{Hz}$, H-5), 7.67 (dd, 2H, $J_{\text{H-2,F}}=15.39\text{Hz}$, H-2, H-6), 8.42 (d, 1H, $J=7.22\text{Hz}$, NH);
20 ^{19}F NMR ($\text{Me}_2\text{SO}-d_6$) δ -123.65(dd);
mass spectrum (FAB) m/z (409 [$\text{M}+\text{H}^+$], 48), 262 (M-glu, 100);
Accurate mass expected 409.0733 found -0.7ppm;
Anal: $\text{C}_{16}\text{H}_{19}\text{N}_2\text{O}_5\text{FCl}_2-0.18\text{TFA}-0.2\text{EtOAc}$ requires C-46.07, H-4.68, N-
25 6.26, F-6.54, Cl-15.85, found C-46.29, H-4.80, N-5.99, F-6.29, Cl-15.99. (The presence of EtOAc and TFA noted in the
elemental analysis were confirmed by NMR).
This compound reacted positively with the Epstein spray reagent.

EXAMPLE 3: ABILITY OF PRODRUGS TO ACT AS CPG2 SUBSTRATES

The ability of the prodrugs to act as a substrates for CPG2 was tested.

The enzyme kinetics were measured as described in Springer et al., Eur. J. Cancer (1991) 27, 1361-1366. The results are shown in Table 1. All the 3-fluoro substituted prodrugs tested (19, 20 and 21) were found to be good substrates for CPG2. Of the 2-fluoro substituted prodrugs, prodrugs (8) and (9) were good substrates, but prodrug (7) was such a poor substrate that it was not possible to measure its kinetics.

TABLE 1**Kinetics of Prodrugs as substrates for CPG2**

<u>PRODRUG</u>	<u>K_m/μmol</u>	<u>k_{cat}/s⁻¹</u>
7	very poor substrate	
8	11	213
9	15	462
19	17	565
20	6	614
21	10	1028

EXAMPLE 4: REACTIVITY OF THE PRODRUGS AND ACTIVE DRUGS

The chemical half lives of the prodrugs and the active drugs were measured in order to determine their relative reactivities.

The half lives were measured in a pH stat, by titrating against NaOH, according to Springer et al, Anticancer Drug Design (1991) 6 467-479. The results are shown in Table 2. All three 2-fluoro prodrugs (7, 8, and 9) and their corresponding active drugs (10, 11 and 12) were deactivated.

The chemical half lives of the 2-fluoro prodrugs were too long to be measured in a pH stat. In contrast, the 3-fluoro prodrugs (19, 20 and 21) and the corresponding drugs (22, 23 and 24) were activated compared to the corresponding non-fluorinated analogues and 2-fluoro analogues.

TABLE 2

Chemical Half-Lives

<u>Prodrug</u>	<u>t_{1/2} (min)</u>	<u>Active Drug</u>	<u>t_{1/2} (min)</u>
7	nd	10	93
8	nd	11	192
9	nd	12	1242
19	9	22	1.9
20	122	23	2.4
21	147	24	72
*25	42	*28	21
*26	984	*29	58
*27	1158	*30	324
nd - not determined		* For comparison	

15 25 = 4-[bis(2-mesyloxy)ethyl]amino]benzoyl-L-glutamic acid

26 = 4-[(2-chloroethyl)[2-(mesyloxy)ethyl]amino]benzoyl-L-glutamic acid

27 = 4-[bis(2-chloroethyl)amino]benzoyl-L-glutamic acid

28 = active drug corresponding to 25

20 29 = active drug corresponding to 26

30 = active drug corresponding to 27

EXAMPLE 5: CYTOTOXICITY OF THE PRODRUGS WITH AND WITHOUT CPG2
IN A COLORECTAL CELL LINE

The 2- and 3-fluoro prodrugs 7-9 and 19-21, and the non-fluorinated prodrug 26 were tested for prodrug activity by measuring their cytotoxicity with and without CPG2 in the colorectal cell line LS174T for 1 h (Tom et al (1976) In Vitro 12, 180-181). The corresponding active drugs 10-12, 22-24 and 29 respectively were screened under the same conditions.

The results are shown in Table 3. All the 3-fluoro prodrugs 19-21 showed substantial prodrug activity as did the non-fluorinated prodrug 26. In each case the prodrug was completely non-cytotoxic even at 800 μ M and conversion to its corresponding drug by CPG2 led to increased cytotoxicity. The cytotoxicity of each of the active drugs 22-24 and 29 alone was not significantly different from that of its prodrug + CPG2 (19-21 and 26) respectively. Although all the 2-fluoro prodrugs alone were non-toxic, none exhibited prodrug activity since they were not converted to a cytotoxic species in the prodrug + CPG2 tests. These data were in good argument with the cytotoxicity experiments using the 2-fluoro active drugs.

TABLE 3

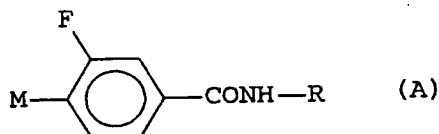
Biological Assay of the Compounds in Cell Culture
with and without CPG2

5	<u>Prodrug</u>	<u>IC₅₀/μM</u>	<u>Active Drug</u>	<u>IC₅₀/μM</u>
	7	>800	10	>800
	7 + CPG2	>800		
	8	>800	11	>800
	8 + CPG2	>800		
10	9	>800	12	>800
	9 + CPG2	>800		
	19	>800	22	480
	19 + CPG2	480		
	20	>800	23	280
15	20 + CPG2	350		
	21	>800	24	270
	21 + CPG2	390		
	26	>800	29	185
20	26 + CPG2	200		

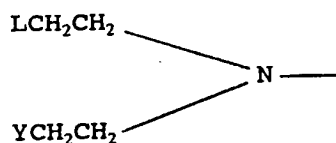
CLAIMS

1. A compound which is a 3-fluorobenzamide of the formula (A)

5



wherein R-NH is the residue of an α -amino acid R-NH₂ or
 10 oligopeptide R-NH₂, and M is a nitrogen mustard group of the formula



15 wherein Y and L, which may be the same or different in a molecule, are leaving groups; or a pharmaceutically acceptable salt thereof.

2. A compound according to claim 1 wherein Y and L, which may be the same or different in a molecule, are selected
 20 from halo, mesyloxy and 4-tosyloxy.

3. A compound according to claim 2 wherein Y and L are both mesyloxy, Y and L are both chloro, or Y is mesyloxy and L is chloro.

4. A compound according to any one of the preceding
 25 claims wherein the amino acid R-NH₂ is glutamic acid or aspartic acid.

5. A compound according to any one of the preceding claims wherein the amino acid R-NH₂ is an L-amino acid.

6. A compound according to claim 1 which is

3-fluoro-4-[bis-[2-(mesyloxy)ethyl]amino]benzoyl-L-glutamic acid,

3-fluoro-4-[(2-chloroethyl)[2-(mesyloxy)ethyl]amino]benzoyl-L-glutamic acid,

- 5 3-fluoro-4-[bis(2-chloroethyl)amino]benzoyl-L-glutamic acid; or a pharmaceutically acceptable salt thereof.

7. A pharmaceutical composition comprising a compound as claimed in any one of the preceding claims and a pharmaceutically acceptable carrier or diluent.

- 10 8. A kit comprising a compound as claimed in any one of claims 1 to 6 or a composition as claimed in claim 7, and an immunoglobulin/enzyme conjugate in which the immunoglobulin is specific for a tumour-associated antigen and the enzyme will cleave the amide bond between the residue of the α -amino acid
- 15 R-NH₂ or oligopeptide R-NH₂ and the benzoic acid nitrogen mustard residue.

9. A compound as claimed in any one of claims 1 to 6, a composition as claimed in claim 7 or a kit as claimed in claim 8 for use in a method of treatment of the human or animal
- 20 body by therapy.

10. A compound, composition or kit according to claim 9 for use in a method of treatment of cancer.

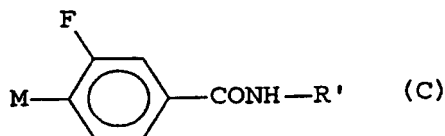
11. A compound, composition or kit according to claim 10 for use in a method which comprises administering to a human
- 25 or animal suffering from cancer pharmaceutically effective amounts of

(i) an immunoglobulin/enzyme conjugate in which the immunoglobulin is specific for a tumour-associated antigen, and the enzyme will cleave the amide bond between the residue of

the α -amino acid $R-NH_2$ or oligopeptide $R-NH_2$ and the benzoic acid nitrogen mustard residue; and thereafter

(ii) the said compound or composition.

12. A process for producing a compound as claimed in
5 any one of claims 1 to 6 and 9 to 11, which process comprises deprotecting a compound of the formula (C)

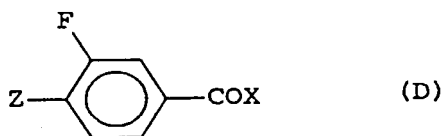


10

wherein M is as defined in claim 1, 2 or 3, and R'-NH is the residue of an α -amino acid R'-NH₂ or oligopeptide R'-NH₂ containing at least one protected carboxylic acid group, and
15 optionally converting the resulting compound of formula (A) as defined in claim 1 into a pharmaceutically acceptable salt thereof.

13. A process according to claim 12 wherein the at least one protected carboxylic acid group is protected by an
20 ethyl or a tertiary butyl group.

14. A process according to claim 12 or 13 wherein the compound of formula (C) is obtained by reacting a compound of formula (D)



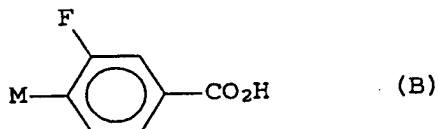
25

wherein X is hydroxy or halo and Z is a group capable of being converted to a nitrogen mustard group M as defined in claim 1,

with an α -amino acid or oligopeptide as defined in claim 12 or 13, followed by conversion of the group Z to a group M as defined in claim 1, 2 or 3.

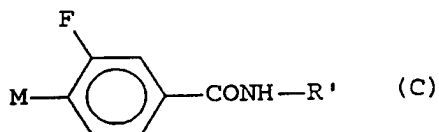
15. A compound of the formula (B)

5



10 wherein M is as defined in claim 1, 2 or 3.

16. A compound of the formula (C)



15

wherein M is as defined in claim 1, 2 or 3, and R'-NH is the residue of an α -amino acid R'-NH₂ or an oligopeptide R'-NH₂ containing at least one protected carboxylic acid group.

20 17. A compound according to claim 16 wherein the at least one protected carboxylic acid group is protected by an ethyl or tertiary butyl group.

18. A method of treating a human or animal patient suffering from cancer, which comprises administering to the
25 patient an effective amount of a compound as claimed in claim 1.

INTERNATIONAL SEARCH REPORT

Int. .onal Application No
PCT/GB 94/00941

A. CLASSIFICATION OF SUBJECT MATTER
IPC 5 C07C237/36 C07C229/60 A61K31/195

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 5 C07C A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	J. MED. CHEM. (JMC MAR, 00222623); 90; VOL. 33 (2); PP. 677-81 CHARING CROSS HOSP.; DEP. MED. ONCOL.; LONDON; W6 8RF; UK (GB) Springer C J et al 'Novel prodrugs which are activated to cytotoxic alkylating agents by carboxypeptidase G2' cited in the application see the whole document	1
A	WO, A, 88 07378 (CANCER RESEARCH CAMPAIGN TECHNOLOGY LTD.; UK) 6 October 1988 cited in the application see claims 15-18	1

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

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Date of the actual completion of the international search

1 August 1994

Date of mailing of the international search report

12. 08. 94

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Authorized officer

Pauwels, G

INTERNATIONAL SEARCH REPORT

International application No.

PCT/GB94/00941

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 18
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claim 18 is directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound.
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

Information on patent family members

PCT/GB 94/00941

Form PCT/ISA/210 (patent family annex) (July 1992)

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